M E M O R A N D U M
Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
Pharmacology / Toxicology Review Memorandum

To: File

From: Evi Struble, PhD

Through: Tim Lee, PhD

Subject: BLA 125284 GTC Biotherapeutics

Contents:

I. Background

ATryn® active ingredient is recombinant human antithrombin alfa (rhAT), expressed in and purified from the milk of transgenic goats integrated with the human gene for antithrombin. The product is also referred to as tgATIII in this review.
ATryn® is formulated with citrate, chloride and glycine. It undergoes nanofiltration and a terminal dry heat treatment step (--b(4)----------) for viral removal/inactivation. The FDP is a sterile lyophilized dosage form containing 1750 international units (IU) antithrombin per vial that is intended for intravenous infusion following reconstitution with 10 mL Sterile Water for Injection.

II. Proposed use and doses

Indication

ATryn® is indicated for the prevention and treatment of peri-operative and peri-partum thromboembolic events in hereditary antithrombin deficient (HD) patients.

Dosage

The dosage is individualized for each patient.
In surgical patients, a usual loading dose (assuming baseline AT activity of 50% and body weight of 75 kg) is 1630 international unit (IU) (or 20-25 IU/kg body weight). A usual maintenance dose (assuming baseline AT activity of 50% and body weight of 75 kg) of ATryn® administered by continuous infusion is 368 IU/h (or 4-5 IU/kg/h). The Maximum Daily Dose (MDD) used so far in the clinic for surgical patients was 236 IU/kg/day or ~34 mg/kg/day.
In pregnant women, a usual loading dose (assuming baseline AT activity of 50% and body weight of 75 kg) is 2885 IU (or 35-40 IU/kg body weight) infused over a period of 15 minutes. A usual maintenance dose (assuming baseline AT activity of 50% and body

weight of 75 kg) administered by continuous infusion is 695 IU/h (or 9-10 IU/kg/h). Maximum Daily Dose (MDD) used so far in the clinic is 260 IU/kg/day or ~37 mg/kg/day.

III. Recommendation

This reviewer identified no issues that would prevent this BLA from being approved.

IV. Key Findings and Conclusion

Toxicology

The highest dose in 14-day repeat toxicity study in monkeys was 300 mg/kg/day or ~8 times MDD. At this dose, female monkeys exhibited liver toxicity and internal bleeding both of which were not seen in males. On day 15 increased AST and ALK were observed in the highest dose animals. The AST increase was likely due to muscle damage associated with injection site reaction; ALK returned to normal by day 22. Hematological findings in females at the highest dose were a decrease of red cells, hematocrit and hemoglobin and an increase in reticulocytes and polymorphonucleocytes. These changes could be due to multiple blood samples collected during the first day of the study; they returned to normal by day 22. NOAEL in monkeys was 36 mg/kg or approximately equal to MDD. A dose of 120 mg/kg/day caused swelling and bruising at administration sites, but no other effects, including no gross necropsy or microscopic findings.

The highest dose of in the 28-day repeat-dose toxicity study in rats was 360 mg/kg/day, ~ 10 times MDD for treatment of HD patients in high-risk situations (~34 to 37 mg/kg/day). The observed toxicity at this dose was transient limb swelling and local injection site bruising/swelling.

The highest dose in the single dose toxicity studies in rats and dogs were 360 mg/kg and 210 mg/kg, respectively. The observed toxicities at these doses were transient swelling observed in rats and dogs at the highest doses tested, and increased AST at highest dose in the dog study, both resolved during recovery period. AST increase was likely due to soft tissue damage due to local injection site reaction.

The highest dose used in reproductive toxicity studies and administered during most of pregnancy was 210 mg/kg/day – i.e. ~6 times MDD (~37 mg/kg/day) in pregnant women. At this dose, a slight, statistically significant decrease in pup viability or an increase in pup mortality - 4% - was observed. The same dose is NOAEL in rats when used around parturition and during lactation. A dose of 21 mg/kg/day or 0.6 times MDD used so far in pregnant women is NOAEL when used during most of the pregnancy in rats.

Pharmacokinetics:

Five pharmacokinetic and three toxicokinetic (1 single-, 2 repeated-dose) studies were performed by intravenous route in mice, rats, dogs and monkeys.

Pharmacokinetic (PK) and Biodistribution (BD) profiles of non-heat treated and nanofiltered heat treated have been analyzed in two studies --b(4)---100 (non-heat treated) and 04-0585P (heat-treated and nanofiltered heat treated). There is no difference in PK and BD of non-nanofiltered and nanofiltered tgATIII. PK parameters are comparable for the non-heat treated (--b(4)---100) and heat treated (04-0585P) animal studies. BD in the rat was generally similar for rhATIII and hpAT. There was one difference in the BD disposition of tgATIII. Namely, there was more drug related radioactivity associated with the gastrointestinal tract following ^{125}I-tgATIII than ^{125}I-plasma derived ATIII administration suggesting higher bile elimination for the recombinant product.

In toxicokinetic rat studies, Area Under Curve (AUC) increased with dose in a non-linear manner. AUC values were greater after 4 weeks of dosing compared with initial values, suggesting accumulation. The clearance for pdATIII was lower when compared to tgATIII at each dose and could be due to the different glycosylation pattern.

AUC measured in toxicokinetic study in --b(4)------- monkeys was 3-4 times greater than in the rat at all doses used. Evidence of accumulation in monkeys was observed at dose levels ≥ 300 mg/kg/day. Other kinetic parameters in the primate underscore the cross-species difference: the clearance in monkeys is slower and the elimination half-life is longer than those measured in the rat model. All PK values in the monkey were comparable with the ones measured in the dog.

Formulation, Excipients:

Formulations used in pre-clinical studies differ from the FDP. The pre-clinical formulations contained non-nanofiltered and non-heat treated antithrombin alfa. The exceptions are the reproductive toxicity studies #--b(4)--007-001 and #6354-13, and bioequivalence study #04-0585P which used the nanofiltered and terminal heat treated product. The nanofiltered and terminal dry heated product has increased aggregation and increased deamidated forms, both within release specification values. These effects have not been evaluated in animals. However, PK similarities suggest similar immunogenicity profiles. The comparability of these lots has been assessed in the clinical PK study (GTC AT PK 011-04).

The composition of FDP, including its excipients, is shown in Table 1, adapted from the submission. All excipients are present in other FDA approved drugs administered via the same ROA and having the same concentration and exposure found in the ATryn® formulation (data obtained from Inactive Ingredients Database and the Physician Desk Reference).

Table 1. Qualitative and Quantitative Composition of ATryn®

Component	Quality Standard	Function	Amount per Vial	Deliverable Amount per Vial (concentration in g/100 mL)
Antithrombin alfa	--b(4)---	Active Ingredient	262.5 mg (1838 IU[a])	NLT[b] 250 mg - 1750 IU
Glycine	--b(4)---	--b(4)---	104.8 mg	100 mg (0.1%)
Sodium Chloride	--b(4)---	--b(4)--- --b(4)---	82.8 mg	79 mg (0.8%)
Sodium Citrate	--b(4)---	--b(4)---	27.1 mg	26 mg (0.26%)
--b(4)---	--b(4)---	--b(4)---	--b(4)---	--b(4)---

[a]Each mg of antithrombin alfa possesses approximately 7 international units (IU) of activity when tested in a thrombin inhibition assay using a house reference standard which has been calibrated against the WHO International Standard for antithrombin. Thus, when reconstituted with 10 mL of diluent as directed each vial contains approximately 1838 IU of antithrombin of which approximately 1750 IU are deliverable from the vial.
[b] NLT: Not Less Than
[c] USP: United States Pharmacopoeia
[d] EP: European Pharmacopoeia
[e] NF: National Formulary
[f] QS: Quantity Sufficient
[g] Not Applicable

V. Comments

Immunogenicity after re-exposure needs to be addressed either in a pre-clinical study or via post-marketing requirement.

Reproductive toxicity studies:

- There is not enough preclinical data to qualify tgATIII as Pregnancy Category A or B under 21CFR Sec 201.57 (f)(6)(i).
- The product can be classified as Pregnancy Category C due to these omissions and conclusions from the reproductive toxicity studies:
    - There are no studies to detect the effect of tgATIII on Female Fertility and Early Embryonic Development to Implantation (See ICH S5A, IV.A.1 (4.1.1)). Both reproductive toxicity studies conducted asses the effect of repeated exposure in premated female rats.
    - There is no embryo-fetal development study in a second non-rodent species (see guidance ICH S5A).
    - Study # 6354-131 shows that there is a slight increase in pup mortality at Day of Lactation 0-4 when a dose of 210 mg/kg/day (~35X loading dose, 5-6X MDD) is administered during most of pregnancy in rats. The same study shows that a dose of 21 mg/kg/day (3.5X loading dose, 0.6X MDD) is safe when used during most of pregnancy. Another study in rats, # AI-007-001, shows the safety of tgATIII up to a dose of 210 mg/kg/day in pregnant rats when used peri-partum and during lactation.
    - Preclinical studies do not assess any potential neutralization of ATryn FDP (nanofiltered and heat treated) due to the immune response. The presence of neutralizing antibodies would make the results of the reproductive studies difficult to interpret and thus needs to be addressed.

VI. List of safety toxicology studies in BLA 125284

Study Type and Duration	Route	Species	Doses (mg/kg)	Study no
Acute Toxicity				
Single-dose toxicity (2 studies)	iv iv	Rat Rat	21-360 36-360	b(4)-3-B68 b(4)-3-E36
Single-dose toxicity	iv	Dog	21-210	b(4)-3-B41
Repeat-dose toxicity:				
28-Day (2 studies)	iv iv	Rat Rat	36-360 36-360	b(4)-3-B42 b(4)-102

14-Day	iv	Monkey	36-360	b(4)-3-B43
Genotoxicity:				
Ames assay	In vitro	Bacteria	0.05-5 mg/plate	b(4)-3-B63
Chromosomal aberration	In vitro	CHO cells	0.5-5 mg/ml	b(4)-3-B65
Micronucleus	iv	Mouse	36-360	b(4)-3-B64g
Reproductive Toxicity:				
35-37 Days	iv	Rat	36-360	b(4)- 6354-13
5-8 Days	iv	Rat	2.1-210	b(4)-AI-007-001

VII. Summary of studies in BLA 125284

Single dose toxicity:

Study b(4)-3-B68
Title: Single dose toxicity study of transgenic goat antithrombin III administered intravenously to Sprague-Dawley rats.
Aim: To evaluate the acute toxicity associated with a single intravenous infusion of transgenic goat antithrombin III (tgATIII) in rats.
Model: Sprague-Dawley rats
Design: Randomized, N=56, 4+1 groups, 5M, 5F / group. The highest dosing group and one additional control group (3M, 3F) were added after the first leg of the study was completed. Animals were euthanized 14 days after test article administration.
Dose: single IV via the tail vein of either vehicle, 21, 70, 210, or 360 mg/kg of tgATIII One mg of ATIII is equivalent to approximately 7 U of thrombin inhibitory activity.
Outcome Measurements: clinical observations 1, 4, and 6 hours after the initial dose on Day 1 and once per day thereafter, body weight and food consumption measurements weekly, and gross pathology.

Results: Transient swelling in the paws, limbs, and face in all animals dosed with 210 or 360 mg/kg. Swelling was resolved between 6 and 24 hours after dosing for all animals.

Reviewer Conclusions and Comments: A single administration of tgATIII was associated with transient swelling in extremities of rats dosed with 210 or 360 mg/kg.

Study b(4)**-3-E36**
Title: A Safety Study of the Interaction of Heparin and tgATIII in Sprague-Dawley Rats

Aim: to evaluate the safety and pharmacologic effect of the combination of heparin and tgATIII when administered consecutively and to compare equivalency between tgATIII and human plasma-derived ATIII (hp ATIII).
Model: Sprague-Dawley rats
Design: 8 groups of 15 rats/sex/group randomized by weight and dosed with the vehicle, glycine citrate buffer, or either tgATIII or hpATIII (Thrombate®) via a 30 minute infusion, followed by a bolus injection of sodium heparin and observed for up to 7 days according to the following schedule. At 10 and 60 minutes and 24 hours after dosing, three rats/sex/group were sampled (blood samples), euthanized and discarded without further evaluation.
3 and 7 days after dosing, three rats/sex/group were sampled (blood samples), and euthanized for comprehensive necropsy with limited tissue collection (gross lesions).
Dose: Dosing levels are shown in table 1. One mg of ATIII is equivalent to approximately 7 U of thrombin inhibitory activity.
Outcome measurements: Live observations twice daily for mortality and moribundity, clinical observations daily, body weights prior to treatment and prior to necropsy. On blood samples the following was performed: activated clotting time (ACT), clinical pathology including hematology (with blood smears), coagulation, serum chemistry and platelet aggregation. Three animals per sex/group euthanized on days 4 and 8 were subjected to a comprehensive gross necropsy.
Data Analysis: Data analysis was performed on body weights and clinical pathology data.

Table 1: Dosing Levels and Study Design

Group number	Number of animals		Treatment administration				Euthanasia
	M	F	Substance	Dose level	route	Dosing regimen	
1	15	15	Glycine/Citrate buffer	0 mg/kg	IV infusion	30 min at 10 mL/kg	3/sex/group at 10 and 60 min and 24 hrs after dosing No necropsy 3/sex/group on Day 4 and Day 8 Comprehensive necropsy
			Sodium Heparin	300 U/kg	IV bolus	Once	
2	15	15	tgATIII	36 mg/kg	IV infusion	30 min at 10 mL/kg	
			Sodium Heparin	300 U/kg	IV bolus	Once	
3	15	15	tgATIII	210 mg/kg	IV infusion	30 min at 10 mL/kg	
			Sodium Heparin	300 U/kg	IV bolus	Once	
4	15	15	tgATIII	360 mg/kg	IV infusion	30 min at 10 mL/kg	

			Sodium Heparin	300 U/kg	IV bolus	Once	
5	15	15	Glycine/Citrate buffer	0 mg/kg	IV infusion	30 min at 10 mL/kg	3/sex/group at 10 and 60 min and 24 hrs after dosing No necropsy 3/sex/group on Day 4 and Day 8 Comprehensive necropsy
			Sodium Heparin	300 U/kg	IV bolus	Once	
6	15	15	hpATIII	36 mg/kg	IV infusion	30 min at 10 mL/kg	
			Sodium Heparin	300 U/kg	IV bolus	Once	
7	15	15	hpATIII	210 mg/kg	IV infusion	30 min at 10 mL/kg	
			Sodium Heparin	300 U/kg	IV bolus	Once	
8	15	15	hpATIII	360 mg/kg	IV infusion	30 min at 10 mL/kg	
			Sodium Heparin	300 U/kg	IV bolus	Once	

Results:
One mortality – group 3 (middle dose) male – no follow up performed, no reason for death given.
Clinical observations associated with tgATIII were transient facial and limb swelling in rats infused with 210 mg/kg or 360 mg/kg within 1 h of administration. Not observed in hpAT dose groups.
No other changes could be related to the administration of the test article.

Reviewer Conclusions and Comments: Study shows safety of the test article in rats. The mortality of 1 rat in the middle dose, in conjunction with the absence of any serious AE in the highest dose group (n=30) seems to be an isolated event, likely not related to test article administration.

Study b(4)**-3-B41**
Title: Single dose toxicity study of transgenic antithrombin III (tgATIII) administered intravenously to Beagle dogs.
Aim: to evaluate the acute toxicity and pharmacokinetics associated with a single intravenous infusion of transgenic antithrombin III (tgATIII) in dogs.
Model: Beagle dogs
Design: N=24, randomized four groups, 3M and 3 F/group
Dose: IV administration of vehicle or test item into right or left saphenous vein and one right cephalic vein at 21, 70 and 210 mg/kg (table)

Text Table 1
Study Design

Group No.	No. of Animals		Treatment Administration				Observation Period
	Males	Females	Substance	Dose Level (mg/kg)	Route	Dosing Regimen	
1	3	3	Vehicle	0	Intravenous infusion of 5.0 mL/kg/hour	Once on Day 1	14 days
2	3	3	tgATIII	21			
3	3	3	tgATIII	70			
4	3	3	tgATIII	210			

tgATIII = Transgenic antithrombin III

1 mg ATIII lot number -b(4)- corresponds to 7 U thrombin inhibitory activity – thus the highest dose is 1470 U/kg.

Outcome measurements:

Clinical observations 1, 4, 6 hrs after administration and daily after, body weight weekly, food consumption daily, ophthalmic examination prior to euthanasia, immunology, clinical pathology. On day 15 gross necropsy, organ weights and selective microscopic evaluation.

Results: Transient increases (2.7-fold) in aspartate aminotransferase in males and females dosed with 210 mg/kg were noted on Day 2, could be associated with skeletal muscle/soft tissue damage suggested by forelimb swelling, not seen on Day 15 and in gross or microscopic observations.

PK: The mean total *AUC* were 137, 694, and 2,889 mgxmin/mL in male dogs and 154, 669, and 3,326 mgxmin/mL in female dogs, for the three doses respectively. Mean $t_{1/2}$ values were 3.3, 5.6, and 5.6 hours in male dogs and 3.6, 4.5, and 8.4 hours in female dogs.

Reviewer Conclusions and Comments:

The study shows safety of tgATIII in a beagle dog model.

Usual causes of AST increase can be liver, cardiac and nephritic necrosis and possibly muscular necrosis. The lack of an increase in a liver-associated enzyme, ALT, in the highest dose subjects showing increased AST levels indicates that liver damage was unlikely. Calcification of tubules and medulla in kidneys was observed in all groups, including vehicle control. There were no gross pathology or microscopic pathology findings for the heart.

Thus, this reviewer concludes that it is very likely these AST changes could be due to muscular damage due to limb swelling. However, this reviewer recommends that in the clinic/clinical trials subjects be monitored for kidney and liver toxicity.

Note 1: Conversations with clinical reviewer, Dr. Jain, revealed that there have been no signs of liver/kidney toxicity observed during the clinical trials.

Note 2: Below is a tabulation of AUC normalized with dose to be used for a comparative PK analysis through different species (presented in pg 14).

Table: Ratio of Mean AUC (mgxmin/mL) with dose (mg/kg) in the dog

Dosing Day	Group 1	Group 2	Group 3

	M	F	M	F	M	F
1	6.5	7.3	9.9	9.5	13.7	15.8

Repeated-dose Toxicity

Study b(4)-**3-B42**
Title: 28 day repeated dose toxicity study of transgenic Antithrombin III (tgATIII) administered intravenously to Sprague-Dawley rats.
Aims:

1. to determine the toxicity of tgATIII after 28 consecutive days of intravenous administration to rats.
2. to evaluate delayed onset of toxicity or reversibility of toxicity after 14-day non-treatment period for control and high-dose animals
3. to evaluate the pharmacokinetics of tgATIII following a single administration and following 28 consecutive days of dosing.

Model: Sprague-Dawley rats
Design: Randomized by weight, 1 group of 10 rats/sex, 2 groups of 8 rats/sex/group, and 1 group of 13 rats/sex. Five toxicology rats per sex in Groups 1 and 4 were held for a two-week recovery period.
The vehicle (Group 1) or the test article, tgATIII, at dose levels of 36,120, or 360 mg/kg (Groups 2-4, respectively) was administered to each rat once daily for 28 consecutive days via a bolus injection into a tail vein (toxicology rats) or via an injection port (PK rats).

Group number	Number of animals[+]		Treatment				Necropsy
	Males	Females	Material	Dose (mg/kg/day)	Route	Dosing regimen	
1	10	10	vehicle	0	IV injection	Once daily for 28 days	5/sex on day 29 5/sex on day 43
2	8	8	tgATIII	36			5/sex on day 29
3	8	8	tgATIII	120			5/sex on day 29
4	13	13	tgATIII	360			5/sex on day 29 5/sex on day 43

[+]Three animals/sex in Groups 2-4 were used for PK study

Dose: IV 0, 36,120, or 360 mg/kg for 28 days.

Outcome Measurements: Clinical observations, body weight and food consumption evaluations, a comprehensive gross necropsy and histopathologic evaluation.

Results: Transient dose related swelling in the snout area and limbs of all rats dosed with 120 or 360 mg/kg within 1 h of administration which decreased over the course of the study and not seen during the recovery period.
There was a statistically significant increase in mean platelet values of females administered 36, 120, or 360 mg/kg rhAT at Day 29 and in the high-dose females at Day 43.
All groups of treated males and the high-dose female group produced antibodies to tgATIII with no relationship of antibody titer to dose administered. Neutralization was not evaluated and no coagulation parameters measured.

Reviewer conclusions and comments:
The study shows safety of the test item after repeated administration in rats.
The biological relevance of the platelet number increase in female rats is unclear. It could be related to the negative feedback regulation of platelet numbers, but the exact mechanism as it relates to thrombin inhibition is not clear.
Neutralization due to antibody formation has not been addressed here, but such a neutralization effect is not observed in any of the other non-clinical studies performed and thus considered unlikely.

Study -----b(4)----------102
Title: 28 day toxicokinetic study of recombinant human Antithrombin III (rhATIII) administered intravenously to Sprague-Dawley rats
Aim: to determine the toxicokinetics of tgATIII after 28 consecutive days of intravenous administration in Sprague-Dawley rats
Model: Sprague-Dawley rats
Design: N=18, randomized in 3 groups of 3M and 3F rats/group dosed daily for 28 days
Dose: IV injection of 36, 120, and 360 mg/kg.

Group Number	Number of Animals		Treatment			
	Male	Female	Substance	Dose level (mg/kg)	Route	Dosing Regimen
1	3	3	tgATIII	36	IV	Once daily for 28 days
2	3	3		120		
3	3	3		360		

Outcome Measurements: Clinical observations, body weights, immunobiology and toxicokinetic analyses.

Results: nose region transient swelling in all three dose levels with some transient limb swelling in the 120 and 360 mg/kg/day animals within 1 h of dosing. Also, retro-orbital blood collection or chromodacryorrhea and other eye changes (opaque, ulceration etc) occurred in the majority of the animals. These findings could be transfusion related and/or an extension of pharmacologic effect.

Antibodies to rhAT after 28 days of treatment were only produced in two of 3 males and one of three females in the 36-mg/kg/day groups. No antibodies were detected in the two higher dosage groups. Neutralization is unlikely based on the un-inhibited C_{max} and AUC at day 28 as measured by thrombin inhibition assay.
AUC (in mgxmin/mL) for 36, 120 and 360 mg/kg is 53.9, 204, and 828 for males and 42.0, 192 and 684 for females respectively on day 1 and 49, 360, and 1,368 for males and 63.5, 360 and 1,296 for females respectively on day 28.

Reviewer Conclusions and Comments:
Based on the low level and the reversibility of adverse events observed this study shows safety of tgATIII after repeated administration in rats.
The kinetic analysis using thrombin inhibition activity assay showed a dose-related increase in C_{max}, AUC_{inf}, MRT and a concomitant decrease in clearance. This could indicate accumulation and saturation of clearance mechanisms.
Below is a tabulation of AUC values normalized by the dose to be used in a cross-species comparison of PK properties of Atryn (pg 14).

Table: Ratio of Mean AUC (mgxmin/mL) with dose (mg/kg) in the rat

Dosing Day	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
1	1.5	1.2	1.7	1.6	2.3	1.9
28	1.4	1.8	3.0	3.0	3.8	3.6

Study b(4)-**3-B43**
Title: 14 Day Repeated Dose Toxicity Study of Transgenic Antithrombin III (tgATIII) Administered Intravenously to ----b(4)---- Monkeys
Aims:

1. To determine the potential toxicity of tgATIII, after 14 consecutive days of intravenous administration to ---b(4)--- monkeys,
2. to determine the reversibility, persistence or delayed occurrence of toxic effects after a seven day non-treatment recovery period, and
3. to evaluate the PK of tgATIII following a single administration and PK following 14 consecutive days of dosing.

Model: ----------*b(4)*---------- monkeys)
Design: N=16 M and 16 F, randomized by weight,
2 groups of 5 M and 5 F and 2 groups of 3M and 3F dosed with either vehicle, glycine citrate buffer (Group 1), or the test article, tgATIII, at dose levels of 36, 120, or 360 (300) mg/kg (groups 2-4, respectively) by a daily one hour intravenous infusion into peripheral vein for 14 consecutive days.

Group Number	Number of Animals		Treatment				Necropsy
	M	F	Material	Dose (mg/kg)	Route	Dosing Regimen	
1	3	3	Vehicle	0	IV	Once daily for	Day 15
	2	2			10		Day 22

2	3	3	tgATIII	36	mL/kg in 1 hr	14 days	Day 15
3	3	3	tgATIII	120			Day 15
4	3	3	tgATIII	360[+]			Day 15
	2	2[++]					Day 22

[+]Dose changed to 300 mg/kg on Study Day 3 for males, Study Day 2 for females.
[++]A Group 4 female was replaced on Day 1 due to its moribund condition caused by inadvertent piercing of the femoral artery.
Dose: 0, 36, 120 and 300 mg/kg.

Outcome measurements:
Clinical observations and food consumption evaluations were performed daily, and body weight evaluations were performed weekly. Hematology, coagulation, clinical (serum and urine) chemistry, antibody measurements using ELISA and thrombin inhibition assay, ophthalmic examinations, electrocardiograms, comprehensive gross necropsy and histopathology were performed.

Results:
NOAEL is 36 mg/kg/day.
120 mg/kg/day caused swelling and bruising at administration sites, but no effects on body weight change, hematology, clinical chemistry, ophthalmology, and gross necropsy or microscopic findings.
In the high dose, 300 mg/kg, 2 unscheduled deaths one due to excessive bleeding likely due to unintentional puncture of the femoral artery the other showing liver necrosis. Also, increase in aspartate aminotransferase (AST) levels in both males and females and alkaline phosphatase (ALK) levels in females.
There were hematology changes namely red cell counts, hematocrits and hemoglobin levels lower than the controls which normalized by day 22.
There was an increase in PT and APTT on Day 8 and 15 in middle and high dose which normalized by day 22.
Antibodies were present in the majority of monkeys at all dosing levels. There was no relationship of antibody expression to dose administered. Neutralizing effect was not evaluated but, the PT and APTT remain elevated at day 8 and 15 and do not fall below control levels at any point, thus neutralization by antibodies does not seem likely.

PK analysis shows accumulation of ATIII for doses > 300 mg/kg
The mean total AUC estimates that were obtained on Study Day 1 at tgATIII doses of 36, 120, and 360 mg/kg were 25, 91, and 441 Uxhr/mL in males and 24, 110, and 434 Uxhr/mL in females, respectively. On Study Day 14, at tgATIII doses of 36, 120, and 300 mg/kg, the mean total AUC estimates were 25, 95, and 394 Uxhr/mL in males and 24, 94, and 374 U-hr/mL in females, respectively.

Reviewer Conclusions and Comments:
This study shows safety of Atryn when administered in monkeys at doses close to human dose. Thus, a dose of 36 mg/kg, or approximately 1X MDD, is NOAEL whereas a dose of 120 mg/kg, 3X MDD, shows only local injection site reversible effects.
However, at high dose tgATIII **female monkeys display hepatotoxicity**: one dead female had multifocal hepatic necrosis with the layer surrounding central veins not

involved and which was bridging adjacent lobules. Also, all the females showed increased AST and ALK levels. This hepatic toxicity could be elimination related. Females also had a higher incidence of bleeding causing one unscheduled death on day one and two instances of bleeding of the uterus, and/or oviducts, vagina, urinary bladder and abdominal peritoneum and muscle. Propensity to bleeding could be considered an extension of pharmacologic action and thus not surprising. However, since the systemic exposure to the drug does not display this dichotomy between male and female monkeys (Table 1 below), it is unclear what the biologic significance of such gender difference in toxicity is. In conclusion, based on the:

1. relative accuracy of this animal model in predicting human response,
2. the similarity of the systemic exposure to the drug between this model and healthy human subjects (see below PK Cross-Species Comparison), and
3. the anticipated use of this product in pregnant females

this reviewer believes it is important that these findings be included in the Package Insert for the product.

PK Cross-Species Comparison:

This reviewer converted the AUC values to comparable units (mgxmin/ml or Uxmin/ml) so that the exposure in all the models presented and in the human trials could be compared. The results are tabulated in the Tables 1-4 presented below. Note that the exposure in monkeys is 3-4 times higher than in rats (study ----b(4)-----**102**). In addition, the AUC in monkeys is comparable with the dog and the human exposure.

Table 1: Ratio of mean AUC with dose [(mgxmin/mL)/mg/kg] in the monkey

Dosing Day	Group 2 (250 U/kg)		Group 3 (840 U/kg)		Group 4 (2520 U/kg)	
	M	F	M	F	M	F
1	5.7	5.7	6.5	7.8	10.5	10.3
14	5.9	5.7	6.7	6.7	11.25	10.6

Table 2: Ratio of Mean AUC (mgxmin/mL) with dose (mg/kg) in the dog

Dosing Day	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
1	6.5	7.3	9.9	9.5	13.7	15.8

Table 3: Ratio of Mean AUC (mgxmin/mL) with dose (mg/kg) in the rat

Dosing Day	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
1	1.5	1.2	1.7	1.6	2.3	1.9
28	1.4	1.8	3.0	3.0	3.8	3.6

Table 4: Ratio of mean AUC with dose [(Uxmin/mL)/U/kg] in the clinical study of healthy male volunteers (GEN/G 9601)

Dosing Day	Group 2 (50 U/kg)		Group 3 (100 U/kg)		Group 4 (150 U/kg)		Group 5 (200 U/kg)	
	M	F	M	F	M	F	M	F
1	6.1	NA	9.9	NA	10.2	NA	10.6	NA

Genotoxicity and Mutagenicity

Study b(4)- **3- B63**

Title: Evaluation of tgATIII in the Ames Assay with E. coli

Aim: To investigate the ability of tgATIII, to induce mutations in Salmonella typhimurium and Escherichia coli using an *in vitro* mutagenesis assay.

Model: Salmonella typhimurium strains ----------b(4)---------- and Escherichia coli - b(4)- strain.

Design: Bacteria were treated with the test material with or without ----b(4)--------- fraction of rat ----b(4)---------- for metabolic activation.

Dose: tgATIII was tested at 5, 1.5, 0.5, 0.15, and 0.05 mg/plate. The negative control was glycine-citrate buffer and the positive controls were 2-anthramine (2-aminoanthracene) for activated experiments -b(4)- and for non-activated conditions depending on the strain ----------b(4)----------.

Outcome measurements: Nr of bacterial colonies growing in restrictive plates as compared to positive and negative controls.

Results: Ames assay for mutagenicity *in vitro* in four different *Salmonella typhimurium* strains and two *Escherichia coli* strains at 0.05 - 5 mg/plate showed no mutagenic potential for tgATIII.

Study b(4)- **3-B65**

Title: Evaluation of tgATIII in the *in vitro* Chromosomal Aberration Assay

Aim: to assess the ability of tgATIII to induce chromosomal damage in cultured Chinese hamster ovary (CHO) cells.

Design: To detect chromosomal damage, cells are arrested at the first metaphase following administration of the chemical. Chromosomes are then analyzed for damage. The CHO cells were treated with vehicle, tgATIII and positive controls with and without rat ----b(4)---------- activation -b(4)- for 3 and 6 hours and then allowed to grow for 22 hrs. The cells were arrested with ---b(4)------ 2 hrs prior to harvest and microscopic evaluation.

Dose: 0.15 to 5 mg/mL

Outcome measurements: chromosomal aberration under light microscope, mitotic index calculated as below:

----------b(4)----------

Results: tgATIII is no different than vehicle in this assay and shows neither genotoxicity nor cytotoxicity up to a concentration of 5 mg/ml.

Study b(4)-**3-B64**

Title: Evaluation of tgATIII in the mouse in vivo micronucleus assay

Aim: To assess the ability of tgATIII to induce micronuclei in the ------b(4)------ polychromatic erythrocytes of --b(4)-- mice.

Model: --b(4)-- mice evaluated for the frequency of micronuclei in polychromatic erythrocytes which is an indicator of *in vivo* genotoxicity.
Design: 4 groups of 5 --b(4)-- mice/ sex were injected intravenously into a tail vein with the vehicle control, glycine citrate buffer, (Group 1) or with 36, 120, and 360 mg/kg/day tgATIII for three consecutive days. One additional group (Group 2) was injected with 50 mg/kg the positive control, ------b(4)---------- once on Day 3. On Day 4, the mice were euthanized and ---b(4)------- smears were prepared for the polychromatic erythrocytes assay.
Dose: 0-360 mg/kg/day

Group Number	Number of Animals		Treatment Administration				Day of Sacrifice
	Male	Female	Test Material	Dose (mg/kg/day)	Route	Dosing Regimen	
1	5	5	Vehicle	0	IV injection	Once/day for 3 days	Day 4
2	5	5	------b(4)-------	50		Once, day 3	
3	5	5	tgATIII	36		Once/day for 3 days	
4	5	5	tgATIII	120			
5	5	5	tgATIII	360			

Outcome measurements: ------b(4)--------- were placed on a slide, fixed, stained and examined for cell morphology.
Data Analysis: Statistical analysis was performed on the frequency of micronucleated PCEs based on -------b(4)----------). The percentage of micronucleated PCEs versus log-dose was evaluated using the Cochran-Armitage trend test with $p \leq 0.05$.
Results:
The test article, tgATIII, did not induce micronuclei in PCEs in either male or female mice and did not reduce the PCE fraction in ---b(4)------- thus was not cytotoxic to developing erythrocytes in -----b(4)----- of these mice when dosed at 36, 120, and 360 mg/kg/day.

Reproductive Toxicity

--b(4)-- Study 6354-131
Title: Intravenous Study for Effects on Pre- and Postnatal Development, Including Maternal Function, in the Rat
Aim: to detect any adverse effects of tgATIII, when administered to rats from implantation through weaning, on pregnant and lactating females and on the development of the offspring.
Model: N=100 premated female -----b(4)-------- rats
Design: Random, four groups of 25 premated females each treated with tgATIII starting on gestation day (DG) 6 through lactation day (DL) 20. This means that tgATIII was administered during approximately ¾ of pregnancy and during the entire lactation period. After weaning on day 21, 20 rats/sex/litter were randomly selected for the F1 postweaning maturation phase study – 7 weeks in duration. The F1 pups were followed

through F1 postmaturation phase /breeding period for a maximum of 21 days, and finally F2 pups underwent observation for 1 day and then sacrificed.
Dose: Control and 2.1, 21, and 210 mg/kg/day of tgATIII were administered to rats iv.
Outcome measurements: Clinical signs, body weight changes, food consumption, and general health were monitored. Reproductive outcomes from dams in the F0 and F1 generations were evaluated. During lactation and pre-weaning, F1 litters were monitored for growth and development, which included the assessment of maturational landmarks, open-field activity, and learning and memory. Necropsies were performed on F0 females, F1 adults, and F1 and F2 offspring.

Results:
F0 - No effect in gestation and lactation. Dose related increase in food consumption in and slightly increased weight gain during gestation.
In F1 lactation: high dose showed increased pup mortality from LD 0 to 4 with 90% viability. This was lower compared to the concurrent controls - 94% viability and historical controls 97.5%. Slight dose related vascular congestion in the stomach area that could be related to increased food consumption. One mortality in middle dose – difficulty during delivery.
F1 - Physical development (body weight increase) of F1 pups and maturation parameters locomotor activity, learning and memory were unaffected by treatment along with subsequent mating (fertility), gestation, and delivery of the F2 pups. Necropsy of F1 and F2 pups showed no remarkable findings attributable to tgATIII administration.

Reviewer Conclusions: Use of 210 mg/kg/day tgATIII from DG 6 to DL 20, i.e. during approximately ¾ of pregnancy and during the entire lactation period in rats results in increased pup mortality in DL 0 to 4, with pups being 4% less viable than the control. There is no effect after DL 4.

-b(4)- Study AI-007-001
Protocol 2307-001
Title: Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of rhAT in Rats, Including Postnatal Evaluations through Day 5
Aims:

1. to evaluate tgATIII under conditions similar to the proposed clinical treatment period (maternal treatment for three days before and continuing for three days after vaginal or Caesarean-delivery of an infant),
2. to evaluate if there is an increased pup mortality for a treatment period of approximately 8 days from DG 20 through DL 4.

Model: N=120 Crl:-b(4)-(--------b(4)----------rats (albino), presumed pregnant.
Design: randomized, four groups, 30 rats/group dosed once daily DG 20 through DL 4
Dose: Buffer, 52.5, 105 and 210 mg/kg/day via intravenous injection
Outcome measurements:
Mortality/moribundity, body weight, clinical observations for edema, feed consumption, and reproductive and postnatal pup observations were monitored. Necropsies were performed on all dams that delivered and nursed their litters and all surviving pups.
Results:

Dose-dependent findings

1. increases in swollen limbs and snouts in the 105- and 210- mg/kg/day dosage groups
2. Increased PT and APTTs in the dams.

Dose independent findings:

1. Perivaginal substance in dams of all doses
2. Chromodacryorrhea in one subject in the high dosage group
3. Stillbirth incidences significantly increased ($p \leq 0.01$) for the 105 mg/kg/day dosage group

Conclusions and Comments: Use of tgATIII in rats during the last 1/10 of the gestation period and first 4 days of lactation is safe for dams and pups.
The dose-dependent toxicities are minor (transient swelling) or an extension of the pharmacologic effect (increased PT and APTT). The dose independent findings are likely due to stress, genetic variability (chromodacryorrhea and still birth increases at middle dose), or not severe.

Reviewer Conclusions and Comments on the Reproductive Toxicity Program:
Based on the reproductive toxicity studies presented, this reviewer has the following comments:

- There is not enough clinical or preclinical data to qualify tgATIII as Pregnancy Category A or B under 21CFR Sec 201.57 (f)(6)(i).
- The product can be classified as Pregnancy Category C due to these omissions and conclusions from the reproductive toxicity studies:
    - There are no studies to detect the effect of tgATIII on Female Fertility and Early Embryonic Development to Implantation [See ICH S5A, IV.A.1 (4.1.1)]. Both reproductive toxicity studies conducted asses the effect of repeated exposure in premated female rats.
    - There is no embryo-fetal development study in a second non-rodent species (see guidance ICH S5A).
    - Study # 6354-131 shows that there is a slight increase in pup mortality at Day of Lactation 0-4 when a dose of 210 mg/kg/day (5-6X MDD) is administered during most of pregnancy in rats. The same study shows that a dose of 21 mg/kg/day (0.6X MDD) is safe when used during most of pregnancy. Another study in rats, #-b(4)--007-001, shows the safety of tgATIII up to a dose of 210 mg/kg/day in pregnant rats when used peri-partum and during lactation.
    - Preclinical studies do not assess any potential neutralization of ATryn FDP (nanofiltered and heat treated) due to the immune response. The presence of neutralizing antibodies would make the results of the reproductive studies difficult to interpret and thus needs to be addressed.

Pharmacokinetic, Toxicokinetic, and Biodistribution Program

(Also see the analysis of studies ---b(4)--102 and 3-B43)

Study #95004

Title: Comparison of the Pharmacokinetics of Three Different Lots of Transgenic ATIII in Mice.

Aim: to compare the pharmacokinetics of three different lots of tgATIII in mice.

Model: -b(4)- female mice were obtained from ----------b(4)----------.

Dose: tgATIII was administered as a bolus tail vein injection at a dose of 6 mg tgATIII/kg body weight.

Sampling: Tail bleeds were performed immediately prior to injection and at t =1, 2, 3, 5, 8, 12, 16, 22, 28, 34, 45, 60, 90 and 120 minutes post injection of the test article.

Serum concentrations of human tgATIII were determined using ELISA -------b(4)----------.

Results: With the exception of the AUMCs ($p = 0.048$), there were no significant ($p \leq 0.05$) lot-to-lot differences in the model dependent and model independent pharmacokinetic parameters describing the clearance of tgATIII lots -----b(4)----------from mouse serum.

Study #95038a

Title: Effect of Dose on the Pharmacokinetics of Transgenic ATIII in Rats

Aim: To determine the effect of dose on the pharmacokinetics in rats of transgenic human anti thrombin produced in goats (tgATIII).

Model: 10 Male Sprague Dawley rats

Dose: a single bolus tail vein injection of tgATIII dosed at 12.5, 41.7, or 125 mg tgATIII/kg (2 rats/dose) or pATIII dosed at 20 mg pATIII/kg (4 rats).

Design: Blood was taken from tail vein at specified times (table below)

Group	Dose (mg/kg)	Rats (No.)	Bleed Schedule (minutes)
tgAT-III	12.5	2	pre-, 1, 2, 4, 6, 8, 10, 20, 30, 45, 60, 75, 90.
tgAT-III	41.7	2	pre-, 1, 2, 4, 6, 10, 20, 40, 60, 80, 100, 120, 140.
tgAT-III	125	2	pre-, 1, 2, 5, 10, 20, 40, 60, 80, 100, 120, 150, 180.
pAT-III	20	4	pre-, 1, 2, 5, 10, 20, 40, 60, 80, 100, 120, 150, 180.

MRT, CL, and Vss show that clearance is dose dependent – slower clearance at higher doses. The clearance of pATIII was slower than that observed for any of the doses of tgATIII examined. The dose effect was examined in the addendum.

Addendum to Study #95038a

Tittle: Effect of Dose on the Pharmacokinetics of Plasma-Derived Human ATIII in Rats

Aim: To determine the effects of dose on the pharmacokinetics of human plasma derived ATIII (pATIII) in rats, and to compare these results with transgenic ATIII.

Model: n = 6 male Sprague-Dawley rats

Dosing: Rats received either an IV (tail vein) bolus dose of 42 mg/kg or 125 mg/kg

Results: when compared with the pharmacokinetics of tgATIII, AUC values were much greater for pATIII; Vss values were similar between the two ATIII forms; CLs values for pATIII are much smaller than tgATIII. These differences in the pharmacokinetics suggest a slower clearance of pATIII and no effect on distribution.

Study #: --b(4)--100

Title: A pharmacokinetic and biodistribution study of radiolabeled recombinant human antithrombin III ([$^{-b(4)-}$]rhATIII) and radiolabeled plasma-derived human antithrombin III ([$^{-b(4)-}$]pATIII) administered intravenously to Sprague-Dawley rats.

The aim: to determine PK and BD of radiolabeled recombinant human antithrombin III ([$^{-b(4)-}$]tgATIII) and radiolabeled plasma-derived human antithrombin III [$^{-b(4)-}$]pATIII)with and without administration of ----------b(4)----------.

Model: Sprague Dawley Rats.-b(4)- at 0.6 mg/kg was used to cause a 20-30% decrease in plasma ATIII levels at 3 hrs post-administration

Design: 8 groups of 3 male and 3 female/group and 2 groups of 1 male and 1 female (Group 9 and Group 18) to verify model induction.

The study consisted of two phases: the pharmacokinetic phase Groups 1-8 and biodistribution phase Groups 10-17.

The test substances, tgATIII or pATIII were administered intravenously once on Day 1 either alone or three hours following treatment with 0.6 mg/kg ----------b(4)---------- groups 3, 4, 7, 8, 12, 13, and 16 and 17.

Whole body perfusions with saline were performed after anesthetization and prior to tissue collection.

Dose: 75 IU/kg (Groups 1-4 and 10-13) and 375 IU/kg (Groups 5-8 and 14-17).

Animals in Groups 9 and 18 were treated with 0.9% saline three hours following treatment with LPS.

Outcome measurements:

PK - Blood samples were collected from the pharmacokinetic animals at specified time points over a 90-minute period and processed for plasma analyzed for ATIII activity by thrombin inhibition.

BD- animals were anesthetized 90 minutes post-ATIII dose, perfused and selected tissues were collected. All samples were analyzed for radioactivity by -----b(4)----------.

Results:

PK:

Cmax: no gender difference or source difference (pd vs tg) of ATIII.

tmax: The mean time at which the maximum plasma concentration (Cmax) was consistently slightly later following dosing with pATIII versus dosing with tgATIII.

AUC: higher for males than for females in each group and 3-6X higher for pATIII than tgATIII.

The t1/2 values ranged from approximately 30 to 170 min. At the 375 units/kg dose half-lives were 3 times higher for pATIII than for tgATIII.

Mean clearance (Cl) tended to be slightly lower for males than for females in each group. Animals dosed with tgATIII exhibited substantially higher clearance than those dosed with pATIII. Pretreatment with -b(4)- generally resulted in slightly lower clearance.

The data is tabulated in tables 7 and 8 below (copied from the submission).

BD – comparison of pd and tgATIII:

Concentrations of tgATIII in terminal blood were substantially lower than pATIII.
Concentration of tgATIII in liver were 2- to 3-fold higher than for pATIII.
Bile elimination of ATIII is higher for tgATIII than for pATIII.

Concentration in other organs was dose dependent: At the 75 IU/kg dose tissue concentrations were generally higher for pATIII than for tgATIII; at 375 IU/kg (~48 mg/kg) the trend was reversed.

Table 7
Mean Pharmacokinetic Parameter ----b(4)----- Equivalents
Following Intravenous Administration of 75 Units/kg pATIII to Sprague-Dawley Rats

Parameter (Units)	Mean Pharmacokinetic Parameter Estimates (Standard Deviation)							
	0 mg/kg LPS				0.6 mg/kg b(4)			
	Group 1 (rhATIII)		Group 2 (pATIII)		Group 3 (rhATIII)		Group 4 (pATIII)	
	Male	Female	Male	Female	Male	Female	Male	Female
T_{max} (min)	0 (0)	0 (0)	2 (3)	2 (2)	1 (2)	0 (0)	3 (6)	1 (2)
C_{max} (U-eq/g)	1.66 (0.16)	1.70 (0.01)	1.78 (0.12)	1.84 (0.58)	1.33 (0.05)	1.23 (0.09)	1.49 (0.12)	1.63 (0.28)
AUC_{last} (U-eq*min/g)	33.6 (5.1)	30.0 (3.1)	106.5 (1.2)	83.1 (3.4)	39.8 (0.9)	28.2 (2.9)	100.6 (5.7)	92.3 (10.7)
AUC_{Inf} (U-eq*min/g)	44.2 (12.1)	44.4 (3.9)	189.7 (15.5)	132.4 (14.2)	44.5 (0.8)	43.4 (4.4)	264.4 (45.2)	194.9 (19.0)
AUC_{Extrap} (%)	21.4 (16.2)	32.0 (10.6)	43.6 (4.9)	36.8 (5.4)	10.7 (1.1)	34.2 (12.8)	61.4 (4.9)	52.4 (6.6)
$t_{1/2}$ (min)	86.7 (74.1)	126.8 (48.6)	77.1 (11.5)	63.4 (8.2)	32.4 (2.7)	117.4 (53.4)	127.0 (19.8)	98.7 (16.7)
V_d (g/kg)	199.0 (120.1)	316.0 (113.4)	45.8 (2.4)	53.1 (2.4)	80.8 (6.8)	291.6 (102.1)	52.4 (0.7)	54.7 (8.2)
Cl (g/min/kg)	1.836 (0.509)	1.749 (0.143)	0.416 (0.038)	0.586 (0.060)	1.717 (0.014)	1.778 (0.187)	0.290 (0.041)	0.386 (0.029)
$AUMC_{Inf}$ (U-eq*min^2/g)	3695 (3983)	4763 (2232)	21013 (4914)	12050 (2963)	1637 (65)	4937 (3171)	49627 (16843)	27769 (6766)
MRT_{Inf} (min)	73.4 (64.1)	105.3 (44.6)	109.9 (16.2)	90.1 (12.9)	36.8 (1.5)	109.8 (59.4)	184.4 (29.8)	141.6 (24.8)

Table 8
Mean Pharmacokinetic Parameters for Test Compound Equivalents
Following Intravenous Administration of 375 Units/kg ---b(4)--- pATIII to Sprague-Dawley Rats

Parameter (Units)	Mean Pharmacokinetic Parameter Estimates (Standard Deviation)							
	0 mg/kg b(4)				0.6 mg/kg b(4)			
	Group 5 (rhATIII)		Group 6 (pATIII)		Group 7 (rhATIII)		Group 8 (pATIII)	
	Male	Female	Male	Female	Male	Female	Male	F
T_{max} (min)	1 (2)	2 (3)	3 (3)	4 (1)	0 (0)	1 (2)	2 (3)	12 (3)
C_{max} (U-eq/g)	8.43 (1.10)	8.00 (0.46)	8.45 (0.29)	7.97 (0.63)	7.44 (0.73)	7.02 (0.65)	8.23 (0.68)	7.05 (0.49)
AUC_{last} (U-eq*min/g)	357.5 (37.3)	335.7 (49.2)	549.8 (26.1)	483.0 (13.7)	401.6 (19.3)	346.2 (7.7)	563.7 (58.8)	484.1 (25.5)
AUC_{Inf} (U-eq*min/g)	427.5 (48.7)	380.4 (66.9)	1261.1 (63.0)	1054.7 (28.1)	614.1 (75.5)	452.8 (30.3)	1795.5 (208.5)	1131.7 (117.2)
AUC_{Extrap} (%)	16.3 (0.9)	11.4 (2.9)	56.4 (1.7)	54.2 (2.5)	34.2 (4.9)	23.3 (4.2)	68.6 (0.9)	57.1 (2.2)
$t_{1/2}$ (min)	35.5 (0.9)	29.0 (3.2)	110.9 (5.0)	101.1 (6.2)	57.9 (8.7)	44.0 (6.2)	168.7 (11.2)	110.5 (12.2)
V_d (g/kg)	44.1 (4.6)	41.4 (2.0)	46.1 (2.0)	50.4 (0.6)	51.2 (0.8)	53.2 (4.2)	49.8 (4.5)	52.5 (0.6)
Cl (g/min/kg)	0.862 (0.109)	1.004 (0.168)	0.288 (0.016)	0.346 (0.018)	0.621 (0.084)	0.843 (0.062)	0.205 (0.024)	0.332 (0.036)
$AUMC_{Inf}$ (U-eq*min^2/g)	21418 (2974)	16136 (4369)	200188 (17078)	154235 (14544)	52084 (13721)	28336 (5178)	435195 (65890)	182101 (35858)
MRT_{Inf} (min)	50.0 (1.3)	41.9 (4.6)	158.6 (7.7)	146.1 (9.8)	83.9 (11.7)	62.2 (7.5)	241.8 (13.7)	159.9 (14.6)

BD General Results:
There was no gender difference in BD.
Expected high concentrations of ATIII in the thyroid due to accumulation of free [b(4)] – represented 1% of total dose.
The highest concentrations of ATIII were pituitary, urinary bladder, liver, spleen, bone marrow, kidney and sometimes ovaries and uterus for F and adrenals for M.
Percent of [-b(4)-lAntithrombin III-Derived Radioactivity Recovered in Tissues:
Blood 19% to 55% of dose, liver (7-24%), skin (3-19%), skeletal muscle (4-13%), bone marrow (4-12%), kidney and adipose each 1 to 3%, GI tract 5 to 17% of the dose, indicating that some of the intravenously dosed radioactivity was being eliminated in bile.

Conclusion: There is higher bile elimination for tgATIII than for pATIII.

<u>Study # 04-0585P – Study not submitted, only summarized</u>
Pharmacokinetics and Biodistribution of Three Different Lots of b(4) -rhATIII
Administered as a Single Intravenous Injection to Female Sprague-Dawley Rats
Aim: to investigate the pharmacokinetics and biodistribution of three lots of tgATIII, administered as a single intravenous (IV) injection.
Model: Female Sprague-Dawley rats
Design: 3 groups, N=8 animals/group
Serial blood samples were taken at pre-dose, 1, 3, 5, 10, 15,30,45,60, and 90 minutes post-dose. At 90 minutes post- dose, animals were sacrificed and perfused with -b(4)-.

Study Design Table

Group	# of animals	Dose mg/Kg	Conc. Mg/mL	Test Article	Dosing Regimen	Sample collection
1	8	50	25	b(4) rhATIII	IV-bolus	Pre-dose, 1, 3, 5, 10, 15, 30, 45, 60, and 90 minutes Tissues were collected at 90 minutes post dose.
2	8	50	25	b(4) rhATIII	IV-bolus	
3	8	50	25	b(4) rhATIII	IV-bolus	

Dose: tgATIII 50 mg/kg IV via the femoral vein cannula

Outcome measurements: ------b(4)---------- to determine ----------b(4)----------. Tissues: adrenal glands (2), brain, eyes, heart, liver, lungs, bladder, thymus, thyroid, spleen, kidneys (2), stomach, duodenum, jejunum, ileum, cecum, colon, rectum, ovaries (2) and uterus.
Results: A statistically significant difference ($p<0.05$) was determined for the $T_{1/2}$ and T_{max} between Group 1 (Lot Number --b(4)--) and Group 3 (Lot Number -b(4)-). Other PK parameters are not different.

Pharmacokinetics of Three Different b(4) -rhATIII Administered as a Single IV Injection to Female Sprague-Dawley Rats

PK Parameters	-b(4)- rhATIII	-b(4)- rhATIII	-b(4)- rhATIII
Cmax (µg/mL)	1117±143	1116±43	1161±191
Tmax (min)	1.33±0.82 *	1.86±1.57	3.00±1.63 *
T ½ terminal (min)	40.36±6.4 *	40.09±3.7	33.00±3.7 *
Cl (mL/min/kg)	0.84±0.19	0.82±0.10	0.86±0.11
AUC (min x µg/mL)	48916±8015	48745±2200	49752±6242
AUC/dose (min x µg/mL/mg/kg)	1244±261	1236±145	1185±157
Rsq	0.99±0.02	0.98±0.01	0.99±0.01

*Statistically significantly different $p<0.05$

Summary of Biodistribution Results:
The statistically significant differences that cannot be accounted for due to animal variability include Lot #--b(4)-- had less distribution to the liver than Lot #--b(4)-- and Lot #-b(4)-, while Lot #-b(4)--- is statistically significantly lower compared to Lot # -b(4)-. Lot #-b(4)----- had less distribution to the brain than Lot #-b(4)---- and Lot # -b(4)- and were statistically significantly lower compared to Lot #-b(4)-.

Note:
Test Article: ---b(4)-----rhATIII - Lot Number: ---b(4)----- – Only terminal heat treated
Test Article: ---b(4)------rhATIII - Lot Number: ---b(4)----– Nanofiltered and heat treated
Test Article: ---b(4)------rhATIII - Lot Number: ---b(4)-----– Only heat treated

Reviewer Conclusions and Comments:
There are no differences in PK and BD of these lots that can be attributed to nanofiltration.

A comparison of the PK data from this study with the non-heat treated lot used in study -b(4)--100 highest dose level (375 IU/kg or ~48 mg/kg) shows similar values for PK parameters (units converted by this reviewer and tabulated below).

	Heat treated and nanofiltered (--b(4)---rhATIII)	Non-heat treated (-b(4)--100 study)
C_{max} (µg/mL)	1,116	1,174
T_{max} (min)	1.86	1.5
$T_{1/2}$ terminal (min)	40.09	32.25
Cl (ml/min/kg)	0.82	0.93
AUC (minxµg/mL)	48,745	49,514

Although the PK data derived pertain to two different studies, the differences between PK values are within the margin of error. In conclusion, it does not seem likely that the heat treatment changes the PK disposition of tgATIII.

3-B87
Title: Single dose pharmacokinetic study of transgenic Antithrombin III (tgATIII) administered intravenously to --b(4)--- monkeys
Aim: PK analysis of tgATIII after a single intravenous infusion to --b(4)---- monkeys
Model: --b(4)--- monkeys (*-----b(4)----------).*
Design: 5 groups, N=1 F monkey/group dosed with tgATIII, via IV. Blood samples were collected from a catheter or femoral vessel at protocol-specified timepoints.
Dose: IV bolus of 10, 21, 70, 210 and 360 mg/kg tgATIII.

Text Table 1
Study Design

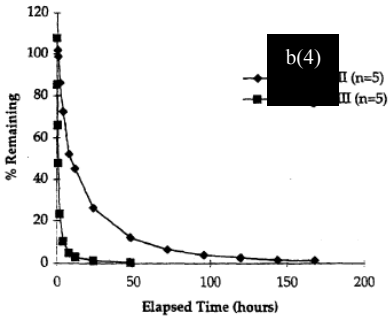
Group Number	Number of Females	Treatment	Dose (mg/kg)	Route
1	1	tgATIII	10	Intravenous infusion at 5.0 mL/kg/hr for 1 hour
2	1		21	
3	1		70	
4	1		210	
5	1		360	Intravenous infusion at 5.0 mL/kg/hr for 1.7 hours†

tgATIII = Transgenic Antithrombin III
† Infusion time was based on a nominal 42 mg/mL stock solution.

Results: The serum AUC values obtained from the total profiles when tgATIII was dosed at 21, 70, 210 and 360 mg/kg were 11, 72.4, 278, and 482 Uxhr/mL.

Other PK parameters of tgATIII are nonlinear at different concentrations with clearance decreasing and half life increasing at higher dose. However, ssVd is independent of dose and equals the plasma volume for the monkey (44.8 mL/kg). At doses higher than 70 mg/kg clearance and half-life values become independent of dose to about 5 mL/hr/kg and 5 hr. This suggests that the nonlinearity may be related to receptors for tgATIII which reach saturation at higher doses.
Reviewer Conclusion: There is evidence of accumulation at higher doses in this animal model.

-b(4)- 2-U93
Title: Pharmacokinetic Evaluation of the Clearance of b(4)-tgATIII and b(4)-pATIII from ----b(4)---- Monkeys Single-dose radiolabeled PK.
Aim: To compare the pharmacokinetics of tgATIII with pATIII in ----b(4)------- Monkeys.
Model: ----b(4)------ monkeys
Design: 2 groups of 5 M/group randomly injected with a bolus of b(4) -pATIII or b(4) – tgATIII.
Dose: trace amounts of b(4) ATIII and carrier protein to a final 3 mg ATIII/kg
Outcome measurements: Blood was analyzed for total cpm and TCA precipitable cpm.



Results: For pATIII - MRT 29.6 hrs and CL 1.9 ml/hr/kg; for tgATIII - MRT 6.9 hrs and CL 13.6 ml/hr/kg.
There is a 4-fold difference in the MRT and a 6-fold difference in CL rates between these molecules with b(4) -tgATIII being cleared much more rapidly from sera than b(4)-pATIII.

Other Studies:
The studies summarized under this section are not GLP compliant. Because GLP safety and pharmacokinetic studies have been conducted by the sponsor and reviewed in this document, the non-GLP studies provided/referenced are of limited use. However, these studies were reviewed by this reviewer; listed below for each study are the study number, primary aim and any observation relating to the safety of the product.

----------b(4)---------- – ----------b(4)----------

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2. ----------b(4)----------
3. ----------b(4)----------.

Study from Referenced Article
Title: Comparison of ATryn and Kybernin Biodistribution in a Rabbit Model
Authors: Leslie R. Berry, Bruce Thong and Anthony K. C. Chan – no number assigned and no signature.
Model – Rabbit
Conclusions: tgATIII is cleared faster than pATIII due to

1. liver uptake using asialoglycoprotein receptor followed by subsequent catabolism, and
2. association with endothelium of arterial/venous surfaces that contain mannose receptors.

The hepatic and vascular surface phases for tgATIII loss may relate to different glycoform populations within the tgATIII product.

Study LMR 8/94
Aim: Efficacy in -b(4)- treated CD Rats.

Study LMR 1/95
Aim: Efficacy in -b(4)-treated CD Rats.

Study KP 1/95
Aim: Efficacy in -------b(4)---------- induced sepsis in rats.

Study 3-B83
Aim: In vitro measurement of fibrinogen levels in blood from rats and humans.

Study 95020
Aim: Explore the dose effect in animal model "sepsis in baboons".
Main observation was increased white blood cell count after test article administration. The effect appears to be due to the buffer alone.

Study 95061b
Aim: Explore the dose effect in baboons.
No increase in white blood cell count was observed after tgATIII administration.

Study 95038b
Aim: Explore the dose effect in rats.
No change in white blood cell count observed after tgATIII administration.